

Comparison of Amplicor and GeneXpert MTB/RIF Tests for Diagnosis of Tuberculous Meningitis

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There are no data about the comparative accuracy of commercially available nucleic acid amplification tests (GeneXpert MTB/RIF and Roche Amplicor) for the diagnosis of tuberculous meningitis (TBM). A total of 148 patients with suspected TBM were evaluated, and cultures served as the reference standard. The sensitivities and specificities (95% confidence interval [CI]) for the Amplicor and Xpert MTB/RIF tests were similar: 46 (31–60) versus 50 (33–67) and 99 (93–100) and 94 (84–99), respectively.

There are ~10 million new cases and 1.7 million deaths from tuberculosis (TB) annually (1). In sub-Saharan Africa and particularly South Africa, up to 80% of TB cases are HIV coinfecting (1) and approximately 40% have extrapulmonary tuberculosis (EPTB). One-tenth of these have tuberculous meningitis (TBM) (2, 3). Patients with TBM frequently require prolonged admission to hospitals and have high morbidity rates due to neuropathology with substantial mortality (~30%), particularly if the diagnosis and follow-on therapy are delayed (4–7). Thus, rapid diagnosis of TBM is essential for early institution of the appropriate therapy. However, the current tools such as smear microscopy perform very poorly in TBM. A systematic review published more than a decade ago showed that nucleic acid amplification tests (NAATs) had an overall sensitivity of 56% and specificity approaching 100% (8).

More recently, however, more sensitive platforms have become available. The Xpert MTB/RIF is a new cartridge-based real-time heminested closed NAAT platform, presently being rolled out in resource-poor settings as a potential easy-to-use point-of-care test (9). The Amplicor PCR is an alternative closed NAAT platform that is now also commercially available (10). We recently reported our experience with Xpert MTB/RIF in TBM (11). However, there are no comparative data evaluating its accuracy in TBM. Here we report our experience using the Xpert MTB/RIF and Amplicor assays side by side, employing cultures as the reference standard.

Methods are outlined only briefly here. More detailed methods are given in the supplement material. A total of 148 consecutive patients with suspected meningitis were prospectively recruited between January 2008 and December 2010. Patients with a meningitic illness who were referred from local district general hospitals were investigated at Inkosi Albert Luthuli Central Hospital (IALCH), a tertiary hospital. Patients had a computed tomography (CT) scan done to exclude contraindications to a lumbar puncture, and blood samples were collected for routine tests, including HIV infection and a CD4 count, and for exclusion of alternate causes of meningitis, including serum fluorescent treponemal antibody (FTA) and Venereal Disease Research Laboratory (VDRL) tests, as previously outlined (12). Cerebrospinal fluid (CSF) was processed for the following tests: microscopy (Gram stain and fluorescence staining for acid-fast bacilli [auramine]), bacterial culture, *Mycobacterium tuberculosis* culture (Bactec 960

MGIT; Becton Dickinson Diagnostic Systems, Sparks, MD), fungal culture, and the cryptococcal latex agglutination test (CLAT). In addition to routine testing, CSF was processed for the Amplicor PCR *Mycobacterium tuberculosis* test (Roche Diagnostic Systems Inc., Branchburg, NJ) (Amplicor PCR) and the Xpert MTB/RIF test (Cepheid, Sunnyvale CA, USA).

Recently archived (–70°C) and uncentrifuged samples ($n = 148$) were processed in an independent laboratory using the Amplicor kit for the detection of *M. tuberculosis*. These samples were used in a previous publication evaluating the Xpert MTB/RIF test for the diagnosis of TBM (11). Here we report on the head-to-head comparison of the two PCR assays, which had not previously been reported. The Amplicor test was done as per the manufacturer's protocol. Briefly, 0.5 ml of CSF was used to extract DNA with the Roche MagNA Pure automated DNA extraction system using a high-performance DNA isolation kit. The extracted DNA was then amplified using the biotinylated primers KY18 and KY75 as described in the kit protocol (Roche Amplicor *Mycobacterium tuberculosis* test). PCR products were detected by the Cobas Amplicor analyzer according to the kit protocol. The samples were also processed for Xpert MTB/RIF analysis at the Lung Infection and Immunity Unit Laboratory (Department of Medicine, Groote Schuur Hospital, University of Cape Town), according to the manufacturer's instructions (13). The laboratory technicians performing the Amplicor PCR and Xpert MTB/RIF assays were blinded to all subject details.

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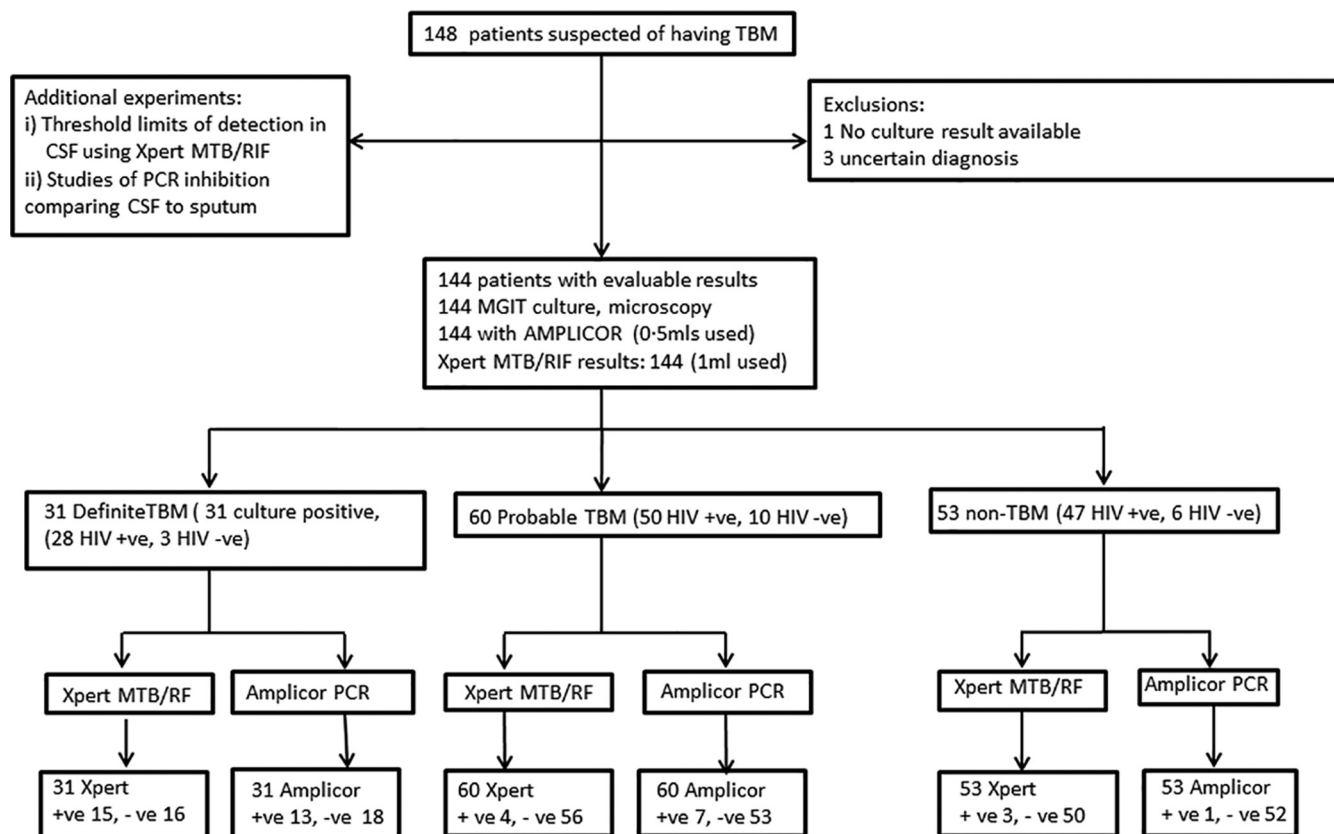


FIG 1 Summary of the study plan, sample processing, and outcome. +ve, positive; –ve, negative.

Patients were categorized, based on standardized published diagnostic criteria, as definite TBM if the CSF *M. tuberculosis* culture and/or the Amplicor PCR test was positive (14, 15), probable TBM (treated empirically with anti-TB drugs but not meeting the definite TBM criteria), or non-TBM (alternate diagnosis confirmed and response to therapy documented in the absence of anti-TB treatment) (16).

The characteristics of definite TBM and non-TBM patients were compared using the chi square test or Fisher's exact test for categorical variables and Wilcoxon's rank sum test for continuous variables. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), overall agreement, and likelihood ratios are reported as measures of diagnostic efficacy. Specificity and sensitivity between the Amplicor PCR and Xpert MTB/RIF tests were compared using McNemar's chi square test. Data were analyzed using Stata v12 (Statacorp, USA).

Figure 1 outlines the study plan and summarizes the sample processing. There were 148 patients tested with the Amplicor PCR and Xpert MTB/RIF assays, but only 144 had both Amplicor PCR and Xpert MTB/RIF results (31 had definite TBM [culture or microscopy positive; identification of the organism was by a niacin/nitrate test for *M. tuberculosis* and by PCR if this was negative], 60 had probable TBM, and 53 were non-TBM). Table 1 outlines the demographic and CSF characteristics that were similar and that differed in the definite TBM and non-TBM groups.

The non-TBM category ($n = 53$) comprised the following breakdown of diagnoses (number of patients): cryptococcal meningitis (29), viral meningitis (13), acute bacterial meningitis (6),

malignant meningitis (2), neurosyphilis (1), parameningeal focus (1), and other (1).

Table 2 outlines the performance outcomes of the Amplicor PCR and Xpert MTB/RIF assays. There were no differences between the Amplicor PCR and Xpert MTB/RIF performance outcomes (sensitivity, $P = 0.7$; specificity, $P = 0.6$; PPV, $P = 0.8$; and NPV, $P = 0.8$). Likelihood ratios were calculated for both the Amplicor PCR and Xpert MTB/RIF assays. The times to detection for the Amplicor PCR and Xpert MTB/RIF assays were 3.5 and 1.5 h, respectively.

There are hardly any data about the performance outcomes of newer NAATs for the diagnosis of TBM. We found no differences in the performance outcomes between the Amplicor PCR and Xpert RIF/MTB assays, and the specificities for both assays were high. The study was relatively large (31 definite TBM cases) compared to those in published reports, where the numbers of culture-confirmed CSF samples were relatively small (17–21). The sensitivity was not improved and remained suboptimal despite our use of fairly large volumes of CSF (500 μ l for the Amplicor PCR and 1,000 μ l for the Xpert MTB/RIF) compared to those in previous studies (22–26). A meta-analysis by Solomons et al. confirmed sensitivities varying from 33% to 67% (27). Previously cited reasons for the suboptimal sensitivity have included the presence of inhibitors in the CSF, paucibacillary samples below the detection limit of the assay, and the aliquot phenomenon (i.e., the initial CSF sample taken may have fewer bacilli than those taken later). We recently found that the degree of inhibition in CSF was lower than that in sputum (11). A single study comparing different aliquots of CSF using PCRs found no differences between the first,

TABLE 1 Clinical and cerebrospinal fluid data from patients with definite tuberculous meningitis (liquid culture or microscopy positive) and non-tuberculous meningitis (culture negative and no anti-TB treatment given)

	Results for persons with:		
Characteristic	Definite TBM (<i>n</i> = 31)	Non-TBM (<i>n</i> = 53)	<i>P</i>
Clinical			
Mean (±SD) age (yr)	32.8 (7.7)	33.1 (11.1)	0.9
Age <36/≥36 yr (no. [%]) ^a	20/11 (64.5/35.5)	34/19 (64.2/35.8)	0.9
Sex, male/female (no. [%])	15/16 (48.4/51.6)	17/36 (32.1/67.9)	0.2
Ethnic group, BA/M/E/I (no. [%]) ^b	31/0/0/0 (100/0/0/0)	52/0/0/1 (98.1/0/0/1.9)	0.9
HIV status, P/N (no. [%]) ^c	28/3 (90.3/9.7)	47/6 (88.7/11.3)	0.9
Previous TB, yes/no/unknown (no. [%])	7/21/3 (22.6/67.7/9.7)	20/31/2 (37.7/58.6/3.8)	0.2
TB contact (within 2 yr), yes/no/unknown (no. [%])	9/19/3 (29.0/61.3/9.7)	14/37/2 (26.4/69.8/3.8)	0.5
Duration of illness, <6/≥6 days/unknown (no. [%])	6/23/2 (19.4/74.2/6.5)	7/45/1 (13.2/84.9/1.9)	0.7
Steroid treatment, yes/no (no. [%])	8/23 (25.8/74.2)	10/43 (18.9/81.1)	0.5
CLAT positive, yes/no (no. [%])	2/27 (6.9/93.1)	26/27 (49.1/50.9)	<0.001
CD4 cells/μl (IQR ^d)	116 (65–196)	161 (78–261)	0.1
CSF parameter (median [IQR])			
Lymphocytes (cells/μl)	117 (24–242)	32 (10–82)	0.004
Neutrophils (cells/μl)	62 (24–138)	9 (0–66)	0.001
Protein (g/liter)	1.7 (1.2–2.5)	1.1 (0.9–1.9)	0.03
CSF glucose (mmol/liter)	1.1 (1.0–1.6)	2.0 (1.5–2.7)	<0.001
CSF/serum glucose ratio	0.2 (0.2–0.3)	0.4 (0.2–0.5)	<0.001
Lymphocytes: total ratio	0.6 (0.3–0.8)	0.8 (0.3–1.0)	0.2

^a This cut point was chosen based on criteria derived by Thwaites et al. (31).^b BA, Black African; M, mixed race; E, European; I, Indian.^c P, positive; N, negative.^d IQR, interquartile range.

second, or third samples taken from the same patient (28). We recently showed that centrifugation of CSF significantly improved the Xpert MTB/RIF assay sensitivity (11). However, we were unable to ascertain whether this also applies to the Amplicor PCR assay as no concentration (centrifugation) experiments were undertaken. This study may have overestimated the sensitivity of NAATs as we used definite TBM (culture positive) as the gold standard. In the probable TBM group, the sensitivity is likely to be considerably lower because the pathogen load is often below the detection limit of the assay (11), as demonstrated in a recent large TBM study by Nhu et al. evaluating the Xpert MTB/RIF assay (29).

The specificity of the Xpert MTB/RIF assay was 94%. This is related to three patients who were classified as non-TBM (culture negative with an alternate confirmed diagnosis but Xpert MTB/RIF positive); two were categorized as having cryptococcal meningitis and one as having leukemic meningitis. Our previous work has shown that such cases (Xpert MTB/RIF positive but culture negative) are likely to be true TB positives (30), as corroborated by the high specificity obtained in large sputum-based studies where a significant minority of the patients had had previous

tuberculosis (11). It is possible that they may have had dual pathologies, but this is difficult to confirm as these patients either died or were lost to follow-up. If these culture-negative, Xpert MTB/RIF-positive persons were hypothetically designated definite TB cases, then the overall case detection rate would have improved by a further $\sim 10\%$.

The limitations of this study include a population restricted to those who were predominantly HIV infected. This may enhance sensitivity, and CSF from such patients may theoretically harbor greater bacterial loads than CSF from immunocompetent patients. Despite the small number of culture-positive samples, this is still a relatively large cohort of definite TBM cases compared to those in similar studies.

In conclusion, this study confirms the modest but equivalent sensitivities for the Xpert MTB/RIF and Amplicor PCR assays in the absence of centrifugation in this predominantly HIV-infected cohort from a country where TB is endemic. Although the sensitivity of the Xpert MTB/RIF assay is modest, it is a useful rule-in test (diagnostic if the test is positive but does not exclude TBM if the test is negative) and thus the Xpert MTB/RIF is useful for the

TABLE 2 Diagnostic accuracy of Amplicor PCR and Xpert MTB/RIF tests using liquid culture and smear microscopy as the reference standard

Test	Performance outcome (95% CI)					LR ^a	
	Sensitivity	Specificity	PPV	NPV	Agreement	LR ⁺	LR [−]
Amplicor	46 (31, 60) ^b 21/46 ^d	99 (93, 100) ^c 80/81 ^d	96 (77, 100) 21/22 ^d	76 (67, 84) 80/105 ^d	80 (72, 86) 101/127 ^d	37.0	−0.55
Xpert MTB/RIF	50 ¹ (33, 67) 18/36 ^d	94 ² (84, 99) 50/53 ^d	86 (64, 97) 18/21 ^d	74 (61, 84) 50/68 ^d	76 (66, 85) 68/89 ^d	8.8	−0.53

^a LR, likelihood ratio. LR⁺ is the ratio of the probability of a positive test among the truly positive subjects to the probability of a positive test among the truly negative, and LR[−] is the ratio of the probability of a negative test among the truly positive subjects to the probability of a negative test among the truly negative subjects.^b Comparison of sensitivity between Amplicor and Xpert MTB/RIF tests: *P* = 0.7.^c comparison of specificity between Amplicor and Xpert MTB/RIF tests: *P* = 0.6.^d An explanation of this fraction is included in the supplementary material.

rapid diagnosis of TBM where delay may otherwise result in excess mortality and significant morbidity. However, studies in other settings, in HIV-uninfected populations, and with variations in processing methods (centrifugation, CSF volume, etc) are now required to improve the sensitivity.

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